Human AHR Antibody

Antigen Affinity-purified Polyclonal Sheep IgG
Catalog Number: AF6185

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human AHR in Western blots.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant human AHR Asn704-Leu848 Accession # P35869
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 μm filtered solution in PBS.

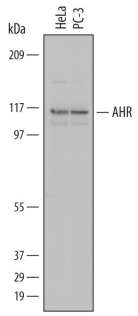
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	1 µg/mL	See Below
Immunocytochemistry	5-15 µg/mL	See Below
Intracellular Staining by Flow Cytometry	2.5 µg/10^6 cells	See Below
Simple Western	10 µg/mL	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	
Knockout Validated	AHR is specifically detected in HeLa human cervical epithelial carcinoma parental cell line but is not detectable in AHR knockout HeLa cell line.	

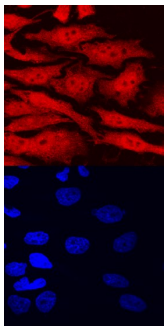
DATA

Western Blot



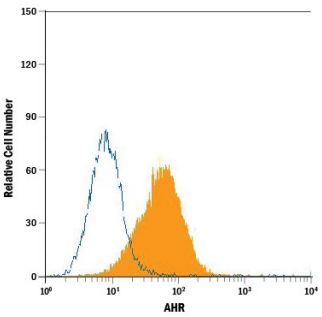
Detection of Human AHR by Western Blot. Western blot shows lysates of HeLa human cervical epithelial carcinoma cell line and PC-3 human prostate cancer cell line. PVDF Membrane was probed with 1 µg/mL of Sheep Anti-Human AHR Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6185) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for AHR at approximately 110 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



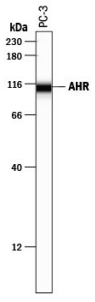
AHR in HeLa Human Cell Line. AHR was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line using Sheep Anti-Human AHR Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6185) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Sheep IgG Secondary Antibody (red, upper panel; Catalog # NL010) and counterstained with DAPI (blue, lower panel). Specific staining was localized to nuclei and cytoplasm. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Intracellular Staining by Flow Cytometry



Detection of AHR in HeLa Human Cell Line by Flow Cytometry. HeLa human cervical epithelial carcinoma cell line was stained with Sheep Anti-Human AHR Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6185, filled histogram) or isotype control antibody (Catalog # 5-001-A, open histogram), followed by Phycoerythrin-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # F0126). To facilitate intracellular staining, cells were fixed with paraformaldehyde and permeabilized with saponin.

Simple Western



Detection of Human AHR by Simple Western™. Simple Western lane view shows lysates of PC-3 human prostate cancer cell line, loaded at 0.2 mg/mL. A specific band was detected for AHR at approximately 109 kDa (as indicated) using 10 µg/mL of Sheep Anti-Human AHR Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6185) followed by 1:50 dilution of HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

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Human AHR Antibody

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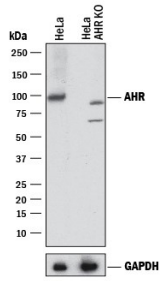
Knockout Validated



Western Blot Shows Human AHR Specificity by Using Knockout Cell Line. Western blot shows lysates of HeLa human cervical epithelial carcinoma parental cell line and AHR knockout HeLa cell line (KO). PVDF membrane was probed with 1 µg/mL of Sheep Anti-Human AHR Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6185) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for AHR at approximately 100 kDa (as indicated) in the parental HeLa cell line, but is not detectable in knockout HeLa cell line. GAPDH (Catalog # 2275-PC-100) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

AHR (Aryl-hydrocarbon receptor; also known as bHLHE76) is a 110 kDa member of the bHLH/PAS transcription factor family. It is widely expressed (breast, lung, liver), and serves many functions. First, it binds multiple xenobiotic chemicals in the cytoplasm. This induces dimerization with ARNT, translocation to the nucleus, and activation of P450 genes such as CYP1A1 and UGT1A6. Second, it appears to block cell cycle progression, possibly via a down-regulation of CDK proteins. And third, it blocks apoptosis by interacting with E2F1, thus silencing TP73 and Apaf1 genes. Human AHR is 848 amino acids (aa) in length. It contains a 10 aa prosegment, plus a 838 aa mature molecule that contains a DNA binding motif (aa 13-40), a bHLH region (aa 41-81), and two PAS domains (aa 111-342). Over aa 704-848, human AHR shares 70% aa identity with mouse AHR.

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